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MECHANISMS OF RADIATION-INDUCED EMESIS

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Canines exposed to 8 Gy $^{60}Co\,\gamma$ mid-abdominal irradiation exhibit emesis whether or not they are subjected to bilateral subdiaphragmatic vagotomy, but do not if they have an ablation of the area postrema. Indomethacin pretreatment reduced the incidence and severity of emesis, and emesis was blocked by pretreatment with domperidone. In electrophysiological studies recording from the area postrema, the chemosensitive neurons were found to be normally silent in anesthetized preparations but excitable by a variety of emetic agents. After irradiation of the abdomen, spontaneously active neurons were found with a discharge pattern that mirrored the behavioral pattern of postirradiation emesis. These studies are consistent with radiation-induced emesis being humorally mediated in canines and implicate dopamine and/or prostaglandins as possible mediators.

In a further effort to identify receptors on the area postrema neurons which might mediate radiation-induced emesis, we have recorded from over 300 neurons and applied various agents

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19. ABSTRACT (Continued)

by ionophoresis. Canine area postrema neurons respond to over 25 substances, including glutamate, acetylcholine, biogenic amines, several peptides and prostaglandins, insulin and calcitonin. In-so-far as they could be tested, there were excitatory responses to substances that were emetic. Glutamate caused rapid discharge of short latency and brief duration, but all other excitatory discharges were of long latency, low frequency and long duration and could be mimicked by application of forskolin or cyclic AMP. Sensitivity for emesis from several agents was increased by pretreatment with phosphodiesterase inhibitors. These results are consistent with the hypothesis that area postrema neurons have specific receptors for a variety of substances, all of which (except glutamate) are coupled to a common adenylate cyclase. It seems likely that the various forms of emesis are mediated by different humoral agents but that the mechanism of excitation is a common one.

PREFACE

We thank Janssen Pharmaceutical for their generous contribution of domperidone which was used in these studies. The assistance of Mr. Laurie Duncan, Mr. Joseph Hutchinson, and Mr. Robert Bochniewicz in dosimetry and irradiaion is greatly appreciated as is the help by Dr. Robert Case in the vagotomy surgery and Mrs. Charlene McAuliffe for secretarial assistance.
This experimentation was conducted strictly according to the principles enunciated in the Guide for Laboratory Animal Facilities and Care prepared by the National Academy of Sciences-National Research Council.

This research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council. Views presented in this report are those of the author; no endorsement by the Defense Nuclear Agency has been given or should be inferred.

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INTRODUCTION

Nausea and vomiting following radiation exposure are factors which may seriously limit the ability of humans to perform in military situations and are side effects of such significance in radiation therapy that they may limit the patient's acceptance of treatment regimes (1). At doses of 1.5 Gy approximately 50% of humans experience nausea and vomiting, while at 3.0 Gy the figure approaches 100% (2,3). While irradiation almost anywhere may produce symptoms, the upper abdomen is the most sensitive site (3). Dogs, cats and monkeys also vomit on exposure to ionizing radiation, although cats and monkeys are considerably more resistant than dogs and man (4).

Our understanding of the neural circuitry of emesis owes much to the work of Wang and colleagues, summarized in Wang's (5) book and in two recent reviews (4,6). A great variety of agents will induce nausea and vomiting, including circulating drugs and toxins, psychosomatic sights, smells and sounds and local gastrointestinal irritation, especially of the stomach. As first demonstrated by Wang and Borison (7), emesis due to circulating substances is dependent upon the integrity of the area postrema, one of the circumventricular organs which lies outside of the blood-brain-barrier and is located bilaterally on the walls of the fourth ventricle. It has been assumed that agents which act here do so through excitatory receptors on the neurons of the area postrema, and that these neurons project to deeper brain stem structures which elicit the motor component of the emetic reflex. In contrast emesis due to local gastric irritation does not depend upon the integrity of the area postrema but rather results from activation of afferent fibers, principally in the vagus, which project to neurons in the nucleus tractus solitarius and dorsal reticular formation. These neurons in the nucleus tractus solitarius probably also receive descending inputs from cerebral regions which mediate psychosomatic vomiting, and are presumed to receive projections from the area postrema. Thus they constitute the motor site of initiation of emesis of all sorts and function as central pattern generators for

the emetic reflex, triggering a sequence and pattern of neuronal activity which activates a great variety of motor neuronal pools independent of the source of the triggering stimulus (6).

There remains considerable controversy over the mechanism of radiation-induced emesis. It is well known that the abdomen is the most sensitive site; therefore it is unlikely that emesis results from a direct action on any central neural structure. In dog and monkey there are reports that radiation-induced emesis is abolished by area postrema ablation (8,9,10,11). In contrast Borison (12) reported that area postrema ablation was ineffective in cats although radiation-induced emesis was abolished by abdominal vagotomy and dorsal rhizotomy of the lower thoracic segments. In the dog the area postrema is also essential for emesis following motion (13) and chemotherapeutic drugs (14).

Thus it appears that the area postrema, which lies outside of the blood-brain barrier (15), has the role of surveying the blood for noxious substances, passing this information on to the motor emetic center which receives direct afferent information and triggers the motor response. Furthermore it is likely, although not proven, that emesis following ionizing radiation results from release of some substance into the blood, and that this substance or substances activate neurons in the area postrema, beginning a cascade of neural activity which ultimately triggers the motor mechanisms of emesis. The identity of this substance is of considerable inportance in the development of rational prophylaxis for radiation-induced emesis.

Because of the small size of the neurons in the area postrema there has been relatively little study of their chemosensitivity. The situation is further complicated by the fact that there are considerable species differences in ability to vomit and susceptibility to vomiting upon various stimuli. Rodents, for example, do not vomit at all (16). Consequently the reports that rat area postrema neurons do not respond to apomorphine, angiotensin and glutamate (17), substances that are

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emetic in man and dog (6) may reflect only a species difference. Cat and monkey do vomit (16), but their sensitivity to drugs like apomorphine is considerable less than that of man and dog. In the one report of attempts to record from cat area postrema, no units were recorded within the area postrema itself, although responses were recorded from deeper structures to application of emetic substances to the surface of the brain stem (18).

The present studies were designed to attempt to determine the roles of the area postrema and the vagus in radiation-induced emesis by ablation and electrophysiological studies, and to test the effects of some drugs on the emetic response. In addition we have recorded from neurons in the dog area postrema, applying substances which may be emetic, in an attempt to determine which transmitters, peptides and hormones might function as chemical mediators of emesis. Finally we have tested the emetic effects of some of these substances given intravenously in awake dogs, with particular emphasis on study of the mechanism of action of emetic agents on the area postrema neurons. Keywords: radiation effects; therapy; vomiting agents; emesis; (RT)

SECTION 2

METHODS

Forty-five conditioned dogs of either sex between 8 and 12 kilograms were purchased from commercial sources, acclimated for one week and maintained on water and dog chow ad libitum. The forelimbs were shaved. For studies in which emetic responses to various substances were tested, the compounds studied were prepared in a physiologic saline solution and were injected i.v. at doses calculated according to body weight. These injections, by protocol, were not more frequently than every other day. Emetic thresholds were determined for the peptides and amines, and a series of three different phosphodiesterase inhibitors were studied and given prior to these injections in an attempt to observe a lowering of the thresholds obtained from the control injections. These animals were subsequently used in the acute experiments described above. Theophylline and IBMX were obtained from Sigma; RO 17624 was obtained from Roche.

Irradiations were performed using a Rotaray Co^{60} therapy unit. Dogs were restrained in a sling, and positioned so that the 4" X 4" port of the unit was centered over the abdomen with the dorsal surface of the back being about 4 cm from the port. A Victoreen Rad-O-Con rectal probe (Model 555) was inserted and centered in the port window to record the radiation exposure. TLDs were also mounted, with three on both back and stomach at the center and extremes of the port, and one on each side and on the dorsal surface of the head. The TLDs were used only to independently confirm exposure and did indeed do so in all experiments. Actual exposure was terminated when the rectal monitor indicated a total exposure of at least 8 Gy, this value being chosen since it has been shown by Gralla et al (19) to give emesis in 100% of dogs. Exposures for the 24 irradiated animals ranged from 7.98 Gy to 8.15 Gy. The rate of irradiation ranged from 0.2 to 0.4 Gy/min, for 15 to 35 min. Although this is a relatively low rate, it is comparable to that used in previous studies in other laboratories (19). In the behavioral studies, the animals were observed for 4 hours postirradiation and the times of productive emesis documented. All irradiated animals were euthanatized by intravenous injection of 1 ml/2.3 kg of T-61 euthanasia solution 4 hr after irradiation. Each ml of T61 euthanasia solution contains 200 mg

of embutramide, 50 mg of mebezonium iodide, 5 mg tetracaine hydrochloride with 0.6 ml of dimethylformamide in distilled water stabilized with 0.0005% sodium bisulfate.

In those experiments in which dogs were both irradiated and studied electrophysiologically, animals were prepared for recording as described below to the point of exposure of the dura over the fourth ventricle, and then were removed from the stereotaxic apparatus to the sling for irradiation. After exposure to 8.0 Gy they were returned to the stereotaxic apparatus, the dura opened and recordings from single neurons of the area postrema made as below.

Lesions of the area postrema were made using aseptic procedures in Nembutal (25 mg/kg) anesthetized dogs, with a ribbon cautery. The subdiaphragmatic vagotomies were performed by Dr. Robert Case, D.V.M., to whom we are grateful. All animals were tested for emesis to 0.025 mg/kg apomorphine prior to surgery to document a normal emetic response. Recovery from surgery was uneventful in all dogs. The animals with area postrema ablation showed some signs of cerebellar irritation (reduced motor coordination) for a few days but these disappeared within one week. Animals were irradiated 90 days after the area postrema ablation and 60 days after vagotomy.

In animals used for electrophysiologic studies, Surital (thiamylal sodium, Parke Davis, 25 mg/kg) was administered as initial anesthesia. An endotracheal tube was inserted providing an access for respiratory assistance if needed. Femoral arterial and venous catheters were surgically implanted for monitoring blood pressure and subsequent anesthesia administration (Nembutal, 25 mg/kg). The animals were mounted in a David Kopf stereotaxic frame and an incision made extending from the occipital ridge caudally approximately 5 centimeters. Bleeding was controlled through the use of an electrocautery. The sterotaxic frame was positioned at a 45 degree angle allowing greater access to and direct visualization of the area postrema. A keyhole incision was made in the bone extending from the occipital ridge to the occipital allantoic membrane. The dura was removed and the cerebellum reflected rostrally to expose the area postrema.

Seven-barreled microelectrodes (R&D Scientific Glass, Spencerville, MD) were used for the recording of extracellular action potentials. The center barrel was filled with 1.0 M NaCl and was used for recording, while the remaining array of 6 barrels were used for ionophoresis of other neurotransmitters. 1 M Na-glutamate (Aldrich-pH 7-8) was always included in the micropipette and was utilized to "find" cells. The electrode was advanced with a Kopf digital microdrive, while concurrently ionophoresing pulses of glutamate in order to elicit an excitatory response from these normally quiescent neurons in the area postrema. The other barrels were filled with various combinations of transmitters which are listed in Table 1. Sources, pH and concentrations of most substances were as previously reported (20). Others include nicotine, pilocarpine and epinephrine all from Sigma and used at pH 3-4, 1M, in saline; neuropeptide Y (Sigma), LHRH (Calbiochem), calcitonin (Peninsula), cyclic GMP (Sigma), 8-bromo-cyclic AMP (Sigma) and theophylline (Sigma) were used at 0.001 M, pH 6-7 in saline; forskolin (Calbiochem) was dissolved in alcohol and diluted to 5% alcohol in saline, pH 6-7 at 0.003 M. Insulin (Sigma 25.5 IU/mg activity), zinc sulfate (Johnson Mattley Chemicals) and α-D(+)glucose (Sigma) were prepared in phosphate-buffered saline with a final pH of 7.2 and applied by electroosmosis using positive current as previously described (20). The prostaglandins, A_1, A_2, B_1, B_2, D_1, D_2, E_1, $F_{1\alpha}$ and $F_{2\alpha}$, were prepared by dissolving in absolute ethanol and diluting to a concentration of 1.4×10^{-3} M (<5% ethanol) in physiologic saline. Stock solutions were stored in a deep freeze at -70 °C.

Upon exposure of the area postrema, a Zeiss OPMI I operating microscope was utilized for visualization and micropipette placement in the transverse center of the structure which in this region is approximately 1000 μm deep. Once tissue contact was made, the digital hydraulic microdrive was set to zero and our protocol allowed penetration within the first 700 μm below the surface, ensuring that our recording was within the anatomical limits of the area postrema and not from deeper structures. The electrode was advanced in 2 μm steps while glutamate was ionophoresed in order to detect neurons. Responses to all the drugs of the ionophoretic array were tested as often as possible and were accepted only if the effect could be repeated.

Table 1. Effects of surgical lesions on radiation-induced emesis.

Lesion	No. of dogs	Emesis after irradiation	Aver. latency (min.)	Aver. episodes	Aver. duration (min.)
None	5	5	102	7.4	96
Area postrema ablation	2	0	-	-	-
Subdiaphragmatic vagotomy	2	2	148	4	100

Data were recorded and stored on a Honeywell Model 1858 U.V. Visicorder paper. Raw traces were viewed using a Tektronix Model 5111 oscilloscope with 5A26 differential amplifiers, and WP Instruments pulse generators and stimulus isolators were used for stimulation. Ionophoretic application of drugs was accomplished through the use of a control module as described by Willis et al. (21), which is a constant voltage source, varying time as opposed to current. Micropipettes were advanced into the tissue with a Kopf digital hydraulic microdrive and extracellular action potentials were recorded with a Dagan Model 2400 preamplifier. Because many spikes generated as a result of ionophoretic application were small (often less than 50 μV) the signals were fed into a World Precision Instruments window discriminator to trigger a pulse output to achieve a better signal to noise ratio.

At the end of the experiment most animals were perfused with saline (0.9%) followed by 10% neutral formalin to verify electrode placement. The brain stems were removed, blocked, embedded and sectioned on a freezing microtome in 20 μm sections and then stained with eosin or hematoxylin for follow-up observation. Animals not perfused were euthanatized by intravenous injection of 1 ml/2.3 kg of T-61 euthanasia solution.

SECTION 3

RESULTS

Table 1 shows results of irradiation studies of control unanesthetized dogs and lesioned animals. As previously reported by Gralla et al. (19), all control dogs vomited after 8.0 Gy exposure, with an average latency of 102 min. These dogs vomited several times over a period of the next 100 min. In contrast neither of the area postrema ablated dogs vomited to a similar irradiation. These two dogs also were resistant to intravenous apomorphine, neurotensin, leucine-enkephalin and angiotensin II as previously reported (Table 3 in Carpenter et al., 22), all of which were emetic prior to surgery. Subsequent histologic analysis showed essentially complete destruction of the area postrema in both dogs, with some mild damage to underlying structures, greater in one dog than the other (Figure 1). In contrast, the dogs with bilateral subdiaphragmatic vagotomy showed no change in their sensitivity to IV apomorphine and vomited after irradiation with a pattern almost identical to that of the controls. These results are most consistent with the view that radiation-induced emesis is humorally mediated through some agent released into the systemic circulation subsequent to irradiation. Although it is known that some vagal afferents project to the area postrema (23,24), the combined results of confirmation of dependency on integrity of the area postrema but independence of the vagus make it very unlikely that any other direct afferent pathway to the area postrema is important.

The next critical question is whether area postrema neurons are excited after exposure to ionizing radiation. This was investigated by recordings made from anesthetized dogs after exposure. In our hands emesis has never been observed in the anesthetized dog, but if radiation caused release of a humoral agent which excited area postrema neurons directly, one might expect that anesthesia would depress the reflex at synaptic sites subsequent to the area postrema. Consequently we searched for spontaneously active neurons in the area postrema after irradiation. Since the nausea and vomiting post-irradiation occurs in a time window 1 to 4 hours after ex-

posure, this is the period in which we would expect to see activity. In studies to date, in which we have recorded activity from over 300 neurons, a spontaneously active neuron has never been observed in the area postrema except on occasions when we caused it to become spontaneously active by ionophoretic application of excitatory substances. Thus, we searched for spontaneous electrical activity post-irradiation.

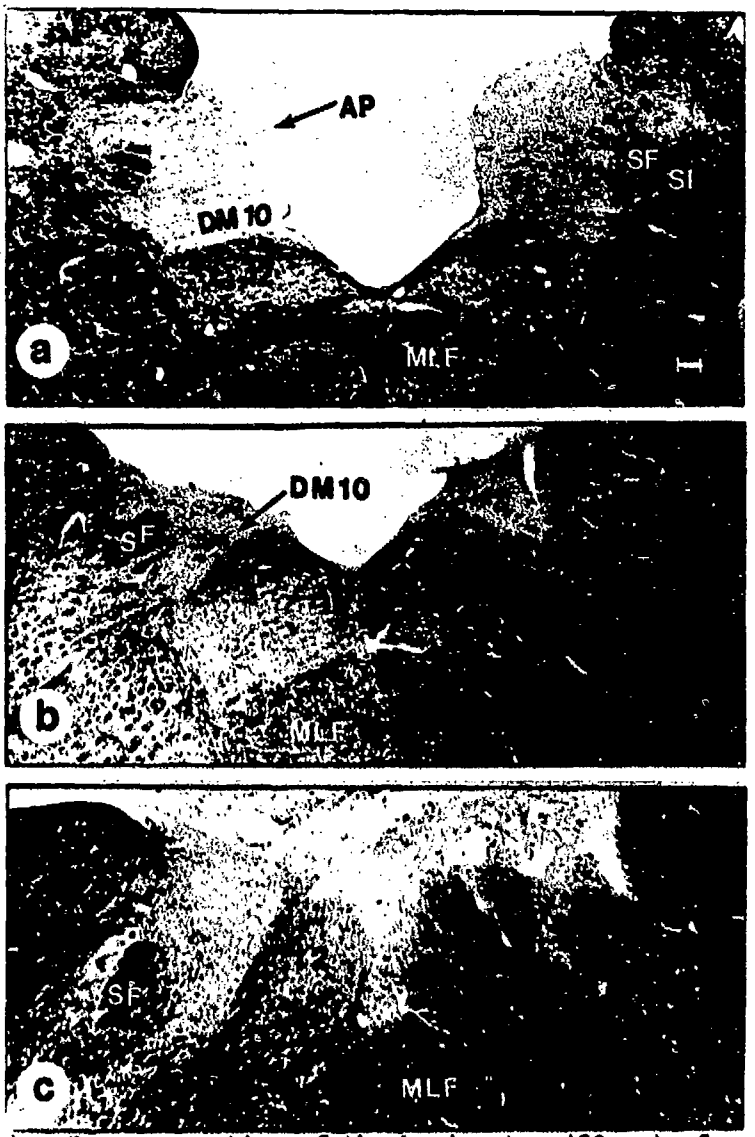

Figure 1. Weil stain of cross section of the brain stem (20 μm) of a control canine (a) and two experimental canines (b and c) subjected to cautery ablation of the area postrema. AP = area postrema, DM 10 = dorsal motor vagus, 12 = hypoglossal motor nucleus; MLF = medial longitudinal fasciculus, SM = medial solitary nucleus; SF = solitary fasciculus; S_1 = lateral solitary nucleus. Calibration is 300 μm.

Figure 2 shows raw data records from a neuron which was found at a depth of 620 μM in the area postrema of a dog 65 min after irradiation. Whereas under every other circumstance neurons were "found" only by pulses of ionophoretic glutamate, this neuron was never exposed to any excitatory substance from the ionophoretic pipette. It was held for an unusually long time, and its discharge accelerated for a period of time, reaching a maximal frequency of about 3 Hz at 175 min, thereafter it slowly decreased in frequency until it stopped about 245 min after irradiation. Thus the spontaneous discharge of this single neuron correlates extraordinarily well with the time course of irradiation nausea and vomiting.

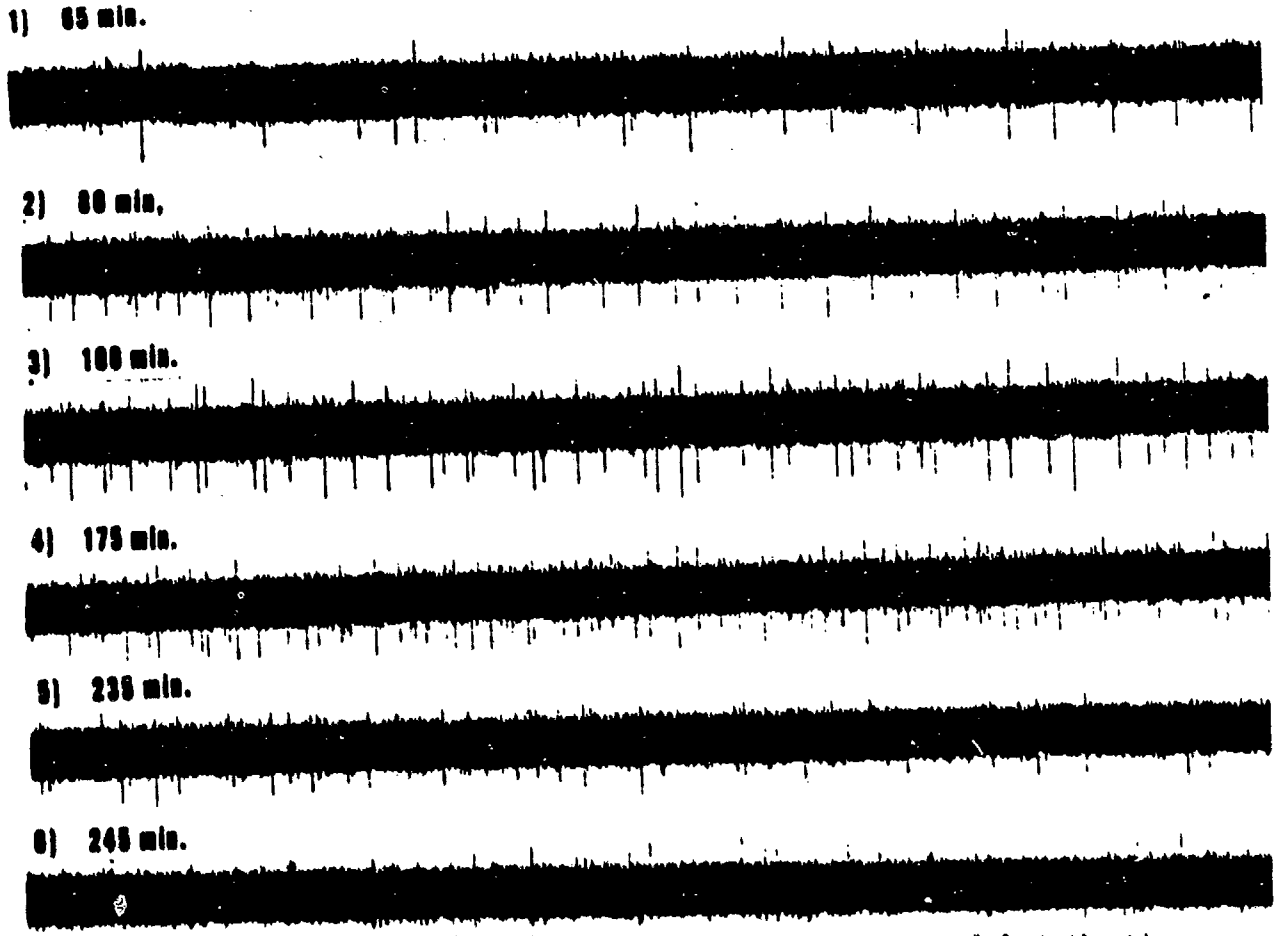

Figure 2. Spontaneous activity of an area postrema neuron recorded at the times indicated after the anesthetized animal was exposed to 8 Gy of Co^{60} irradiation. Record 1 was taken when the unit was "found" by advancing the microelectrode to a depth of 620 μm from the area postrema surface. Without moving the pipette and without any application of drugs the unit was recorded for the next 3 hours.

A total of five spontaneously active neurons were found in the area postrema in two such experiments. Thus we conclude that radiation-induced emesis is associated with and probably mediated through an excitation of area postrema neurons.

The next question concerns the humoral mediation of radiation-induced emesis. Since area postrema neurons are excited by a variety of peptides and neurotransmitters (20), there are many possible candidates. We have pursued two leads using the drugs indomethacin and domperidone.

Indomethacin is an inhibitor of prostaglandin synthesis. It is known that ionizing radiation, along with a variety of other kinds of tissue injury, result in an increase of prostaglandin levels in tissue, (25-27) and urine (28). Moreover, we have found that area postrema neurons are excited by prostaglandins $F_{1\alpha}$, $F_{2\alpha}$, E_1, B_1, B_2, A_1 (see below). If prostaglandins play a role in mediating radiation-induced emesis an inhibition of their synthesis should reduce the incidence of post-irradiation emesis.

Table 2 shows results of irradiation of 7 dogs with indomethacin prior to irradiation. Two dogs did not vomit; in addition the number of emetic episodes was less in the indomethacin-treated dogs which did vomit than in the controls, all of which showed emesis.

It has been reported that domperidone, a D-2 dopamine receptor antagonist which does not cross the blood-brain-barrier (29), blocks radiation-induced emesis in dogs (30) and is therapeutically beneficial in man (31). In order to be sure of the time course of action of domperidone following a single injection of 1 mg/kg 30 min prior to irradiation, we tested the response of dogs to 0.015 mg/kg apomorphine administered every 30 min in 4 dogs before and over a period of 4 hours after administration of domperidone. The apomorphine response was totally blocked for the 4 hour period. Consequently we tested domperidone at 1 mg/kg 30 min prior to irradiation on 4 dogs. None of these dogs vomited after irradiation.

Table 2. Effects of indomethacin and domperidone or radiation-induced emesis.

	Control	Indomethacin (10 mg/kg 30 min prior to exposure)	Domperidone (1 mg/kg 30 min prior to exposure)
Total no. of dogs	5	7	4
No. of dogs showing emesis	5	5	0
Latency:			
Aver. of those vomiting	102 min	115 min	-
Range of those vomiting	40-150 min	71-195 min	-
Duration:			
Aver. of those vomiting	96 min	77 min.	-
Range of those vomiting	69-180 min	59-100 min	-
No. of episodes for those vomiting:			
Average	7.4	3.8	
Range	5-12	1-6	

Domperidone is known to cross blood-brain-barrier poorly (29) and also is known to block apomorphine-induced emesis but not that to several peptides (22). Thus this effect of domperidone is consistent with an action at dopamine receptors in the area postrema. We irradiated two anesthetized dogs given domperidone in order to search for spontaneously active neurons in the area postrema. While we recorded from 8 area postrema neurons in these two animals none were spontaneous; all had to be found by ionophoretic application of glutamate.

In studies investigating responses to ionophoretic applications of agents a total of 308 area postrema neurons have been studied in the dog, and the summary of the excitatory response obtained from these neurons is given in Table 3. This table is an update of the results previously presented on units in 1983 (20). While we have found a few inhibitory responses, notably to histamine and norepinephrine, we have not studied them systematically since the neurons are silent at rest and inhibition can be seen only as depression in the response to other substances, such as glutamate.

Figure 3 shows a raw data record of responses of one neuron to glutamate, forskolin and insulin. This unit, a relatively large one, had extracellular spikes of about 50 μV. While such a recording is clearly distinguishable from the baseline, it does not make a very attractive picture and consequently we have used a window discriminator and raster display to illustrate most of our data, as shown in Figure 2. While there is some peril in using window discriminators, we have recorded and stored both the raw traces and the output of the raster display on photosensitive paper for careful analysis. Responses of many units could be heard on the audiomonitor rather than seen in either the raw data trace or the raster display, but such units were never counted. If the neuron could be seen on the raw data trace, we could usually pick it off within the window with confidence.

Table 3. Transmitter actions on area postrema neurons.

Substance	No. units	% excitation	Reference for emesis due to substance
Glutamate	308	99	(97)
Acetylcholine	15	38	(98)
Nicotine	6	0	
Pilocarpine	13	61	(98)
Serotonin	32	66	(99)
Norepinephrine	15	40	(100)
Histamine	45	64	(101)
Epinephrine	22	32	(102)
Apomorphine	28	71	(103)
Dopamine	11	73	(104)
Insulin	90	54	(32)
Zinc	14	0	
Glucose	16	0	
Angiotensin II	73	47	(22)
Neurotensin	20	25	(22)
TRH	64	66	(22)
VIP	39	46	(22)
Gastrin	11	36	(22)
Substance P	23	48	(22)
Vasopressin	10	50	(22)
Leucine Enkephalin	40	53	(22)
Somatostatin	10	0	(22)
CCK	19	26	
LHRH	2	0	
Neuropeptide Y	4	50	
Calcitonin	8	50	

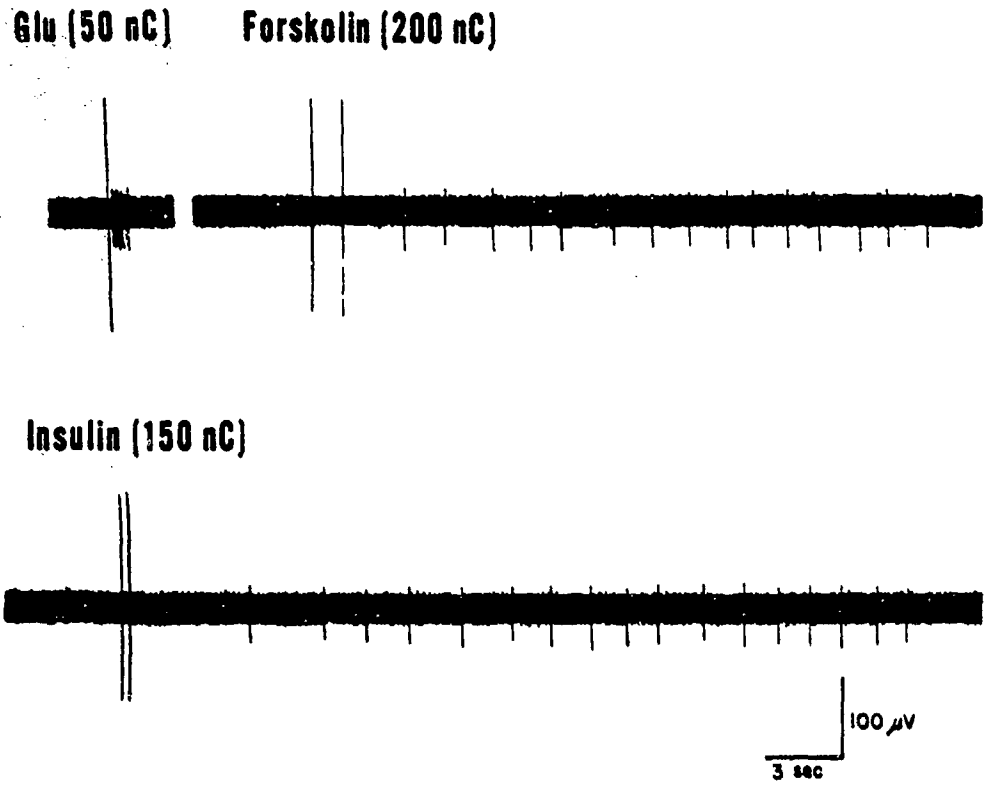

Figure 3. Raw data trace recording from a canine area postrema neuron 536 μm below the surface. Large vertical deflections are ionophoretic artifact, but smaller deflections are action potentials. Glu = glutamate.

Figure 4 shows records from another area postrema neuron and its responses to glutamate, leucine enkephalin and apomorphine. In each application the larger deflection is the ionophoretic artifact, while the unit discharges are represented by the smaller deflections occurring throughout the trace. When glutamate was applied as a brief pulse the neuron discharged with a very brief latency, at a frequency too high to resolve at this sweep speed and with a time course such that the discharge ceased within 1 sec. In contrast, the response to leucine enkephalin at the same ionophoretic current had a latency of about six seconds, a relatively low and erratic discharge frequency and the response lasted for about 30 sec. Such responses could be obtained repeatedly from this neuron but only if not applied at too frequent intervals, since if ionophoresed more frequently than about every five minutes the

response was depressed, presumably due to transmitter receptor desensitization. The middle trace shows that if an increased ionophoretic current of leucine enkephalin is applied, in this case 7 min after the first, a larger response is obtained, characterized principally by being of a longer duration. This neuron also responded to apomorphine ionophoresis, and the apomorphine response had the characteristics similar to that of leucine enkephalin in being of long latency, low frequency and long duration.

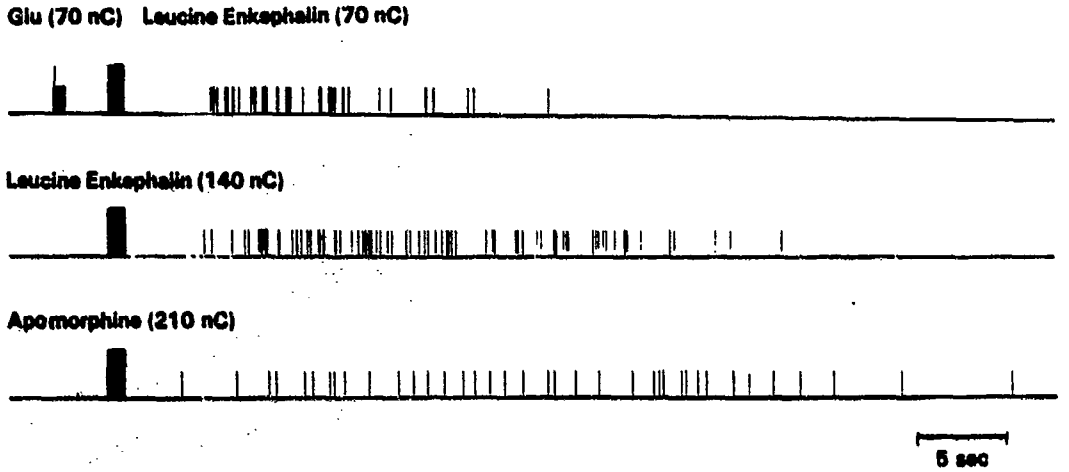

Figure 4. Raster display of responses of an area postrema neuron 502 μm below the surface to ionophoretic application of glutamate (Glu), leucine enkephalin and apomorphine. The time of ionophoretic application is indicated by the large upward deflection, which the smaller deflections are electronic pulses triggered by single action potentials detected through use of a window discriminator. Note the dose-dependency of discharge to leucine enkephalin. There was a 5 min interval between the two leucine enkephalin applications to avoid receptor desensitization.

The results of application of 26 substances on area postrema neurons are shown in Table 3. In addition to glutamate, which was used to "find" the cells, excitation was seen to the biogenic amines, numerous peptides and two hormones. Next to glutamate the highest percentage excitation was seen with dopamine and apomorphine, which are presumed to act at the same receptor. Area postrema neurons were also excited by serotonin, histamine, epinephrine and norepinephrine about half of the

time, and at least for histamine and norepinephrine these responses are complicated in that some neurons show inhibitory responses to these substances, as mentioned above. Thirty eight percent of the neurons studied responded to acetylcholine with a response similar to that illustrated for leucine enkephalin and apomorphine. When tested against nicotine and pilocarpine, specific agonists for the nicotinic and the muscarinic acetylcholine receptors, respectively, there were no responses to nicotine and 61% of the neurons tested with pilocarpine were excited. These results suggest that there are muscarinic but not nicotinic receptors on these neurons.

A number of peptides were applied, chosen principally on the basis of whether or not the peptides were emetic (see Discussion). Excitatory responses were obtained from angiotensin II, neurotensin, thyrotropin releasing hormone, vasoactive intestinal polypeptide, gastrin, substance P, vasopressin, leucine enkephalin, cholecystokinin, oxytocin and neuropeptide Y. Somatostatin and LHRH were chosen for study because our previous results showed both not to be emetic (22) and both were not excitatory. Two hormones excited area postrema neurons, insulin as reported elsewhere (32) and calcitonin. As seen in Figure 3 the response to insulin is like that to these other agents. As controls for the insulin response zinc and glucose were applied, but neither excited the cells.

We have previously reported (22,33) a correlation between excitation of area postrema neurons by a particular substance and behavioral emesis in dogs given an intravenous injection of that substance. In the present study insulin, like apomorphine and leucine enkephalin, was emetic, and the frequency of emesis was dose-dependent (Figure 5). Vomiting has been well documented to result from either of two mechanisms of stimulation, that being direct activation of neurons of the area postrema or local afferent irritation of the gastrointestinal tract. Substances injected iv are unlikely to act on gastric afferents, and the fact that insulin is emetic and excites area postrema neurons can be taken as proof that the area postrema is its site of action, consistent with previous studies (20,22,34). No attempt was

made to control or monitor hypoglycemia; however, the emetic response to insulin was similar to that to apomorphine and leucine enkephalin. For all three the latency of response was about 1 min and emesis occurred 1-3 times over a 2-6 min period. These observations are consistent with the conclusion that insulin induces emesis by activation of area postrema neurons. In two dogs with area postrema ablations no emesis was observed in eight tests at doses which showed greater than 50% emesis in controls. Thus the insulin effect is mediated through the area postrema.

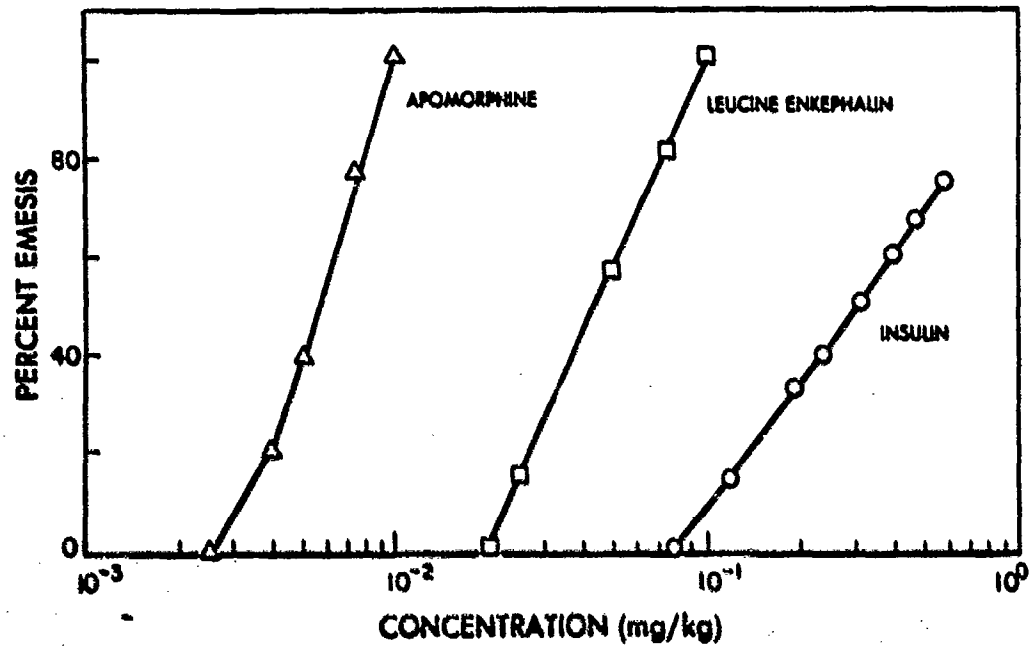

Figure 5. Dose-response relations for emesis induced by intravenous administration of apomorphine, leucine enkephalin, and insulin. For each animal at each dose, emesis either occurred or did not occur. Each data point shows the mean percent of animals affected in multiple trials (apomorphine 10 to 28 trials, leucine-enkephalin, 6 to 22 trials; insulin, 3 to 53 trials). Data for each point are from tests of different dogs and sometimes multiple tests of some dogs. For each substance there was a clear threshold, and for apomorphine and leucine enkephalin there was a dose at which all animals vomited.

Because of the suggestion from the irradiation experiments above that prostaglandins might be mediators of radiation-induced emesis we applied several prostaglandins to neurons of the area postrema. The results are shown in Table 4 and illustrated for one neurons in Figure 6. Several of the prostaglandins tested excited area postrema neurons. Like other substances the responses to prostaglandins had a long latency, a long duration, and a very slow discharge frequency of approximately 4 Hz. Figure 6 shows that the response to glutamate was a high frequency discharge of short latency and duration. Ionophoretic application of prostaglandin E_1 resulted in a response very similar to that of prostaglandin B_2 being long in latency and duration and of relatively slow frequency. Prostaglandin $F_2\alpha$ was applied to the same cell and produced a similar response. Prostaglandin D_2 (150 nC) gave no response and served as an ethanol control.

Table 4. Prostaglandin actions on area postrema neurons.

Substance	No. of units	% excitation
Prostaglandin A_1	15	40
Prostaglandin A_2	12	0
Prostaglandin B_1	17	24
Prostaglandin B_2	21	42
Prostaglandin D_1	12	0
Prostaglandin D_2	14	0
Prostaglandin E_1	15	47
Prostaglandin $F_1\alpha$	23	26
Prostaglandin $F_2\alpha$	16	5

The responsiveness of area postrema units to nine prostaglandins is shown in Table 4. All neurons tested were excited by glutamate which reflects the fact that glutamate excitation was used to find these normally quiescent neurons. Of the nine prostaglandins tested, six were excitatory and three had no effect.

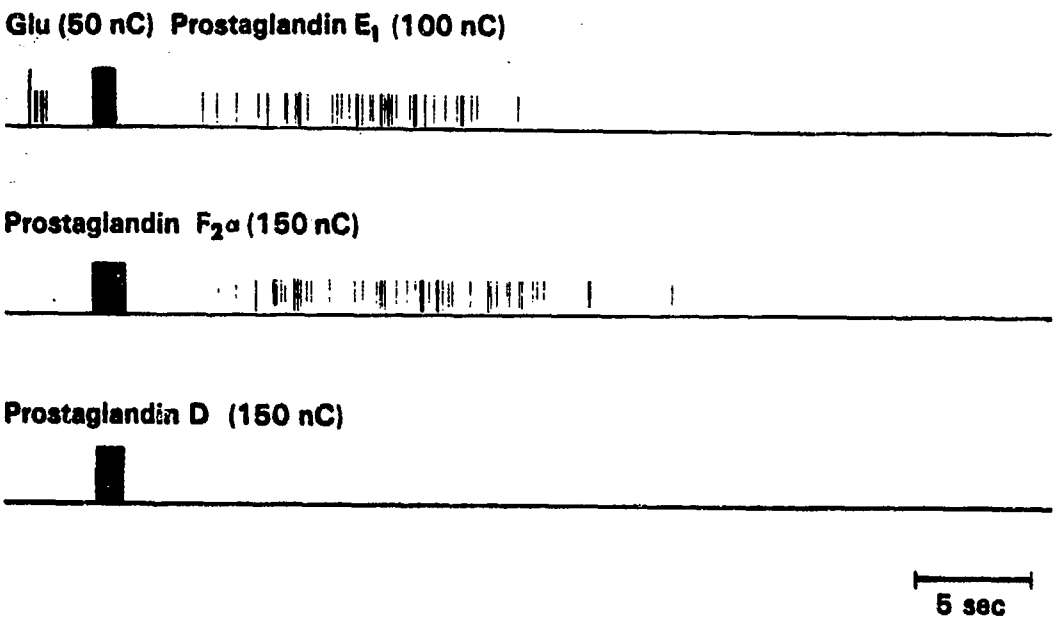

Figure 6. Responses of a neuron 660 μm from the surface to glutamate and prostaglandins E_1, $F_2\alpha$, and D_2. On these traces, the black box is the artifact generated during ionophoretic application, while the smaller vertical deflections are electronic signals reflecting the spikes elicited by the single neuron. Note the rapid, brief response to glutamate in contrast to the more prolonged response to the active prostaglandins.

In summary of these results, these small neurons show excitatory responses to 21 substance shown in Table 1, as well as to several prostaglandins. For all these excitatory responses there is the same relatively long latency, low discharge frequency and long duration.

It is perhaps surprising that such small cells have so many receptors. There are at least three possible explanations for the similarity of responses to so many substances:

1. These neurons carry a single type of receptor which responds to many different substances.
2. There are specific receptors but most act through a common second messenger and thus give a common type of response.

3. There really are a very large number of specific and independent receptors on these neurons, but each is coupled to the same type of ion channel.

Our evidence to date on the specificity of receptors has been limited to study of the behavioral response to activation of these receptors, which is emesis. We have been able to distinguish specific receptors for apomorphine, leucine enkephalin and angiotensin II by use of specific antagonists and by lack of cross receptor desensitization (22). While we have not applied specific antagonists in ionophoretic experiments, it seems unlikely that we are dealing with a relatively nonspecific receptor. It obviously does not lack total specificity, since no responses were found for nicotine, somatostatin and LHRH. Furthermore, our previous antagonist studies have shown specificity in antagonist actions (22).

In order to test the possibility that a common second messenger system is the explanation for the similarity of response to so many substances we applied 8-bromo-cyclic AMP and cyclic GMP, as well as forskolin, an activator of adenylate cyclase (35). Table 5 shows the results of these studies, and Figure 7 illustrates positive responses to forskolin and cyclic AMP. The majority of cells were excited by 8-bromo-cyclic AMP, a derivative of cyclic AMP which crosses the plasma membrane with relative ease. Forty percent of neurons also were excited by forskolin, but excitation was not seen to either cyclic GMP or theophylline, which is an inhibitor of phosphodiesterase. The responses to cyclic AMP and forskolin were similar in appearance to those of the other substances in being of long latency, low frequency and long duration. These results are compatible with the possibility that the actions of all of the 21 excitatory substances which trigger the slow excitations are all mediated with a common second messenger, cyclic AMP.

Table 5. Effects of cyclic nucleotides and related substances.

Substance	No. units	% excitation
8 Bromo-cAMP	48	56
c-GMP	9	0
Forskolin	40	40
Theophylline	10	0

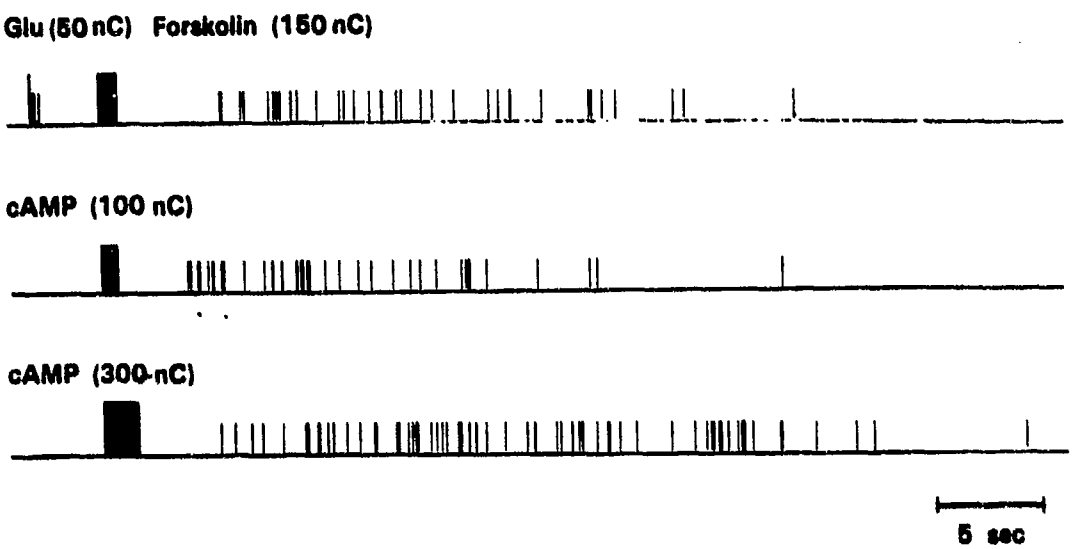

Figure 7. Raster display of responses of an area postrema neuron 620 μm below the surface to glutamate (Glu), forskolin and cyclic AMP. The tall and/or thick black areas indicate time of ionophoresis.

Because of the limitations of our preparation we have attempted to test the possibility that cyclic AMP is a common second messenger by behavioral pharmacology

studies. As we have previously shown (22,32), emesis from intravenous administration of apomorphine, insulin, angiotensin II and leucine enkephalin is dependent upon the integrity of the area postrema, presumably upon those receptors which are described above. Moreover, we have found that one can determine a dose-response curve of sorts to these substances in awake dogs, in that the threshold concentration at which the dog will vomit is very reproducible even when comparing different animals.

Given these facts we designed experiments in which we attempted a total-body inhibition of phosphodiesterase by systemic administration of relatively high doses of theophylline, IBMX and RO-1724, and we determined the emetic potency of selected concentrations of the above emetic substances before and 30 min after administration of the phosphodiesterase inhibitor. Since the area postrema is outside of the blood-brain barrier we would expect systemic administration of these substances to be effective there. If apomorphine, insulin, angiotensin II and leucine enkephalin excite area postrema neurons by stimulating the synthesis of cyclic AMP, the administration of a phosphodiesterase inhibitor should retard inactivation of the cyclic AMP and the animal should respond with emesis to a dose lower than that which caused emesis in the control.

Tables 6, 7 and 8 show results on three different sets of dogs with the three phosphodiesterase inhibitors studied. In each experiment three doses of the emetic agent were chosen on the basis of previous experience to be just below or at the threshold for emesis. Animals were tested not more frequently than once every other day. After controls were obtained, the animals were pretreated with the phosphodiesterase inhibitor and tested with the emetic agent 30 minutes later. As seen in the tables for all three phosphodiesterase inhibitors and for all four emetic agents there was a shift in the dose response curve such that a previously ineffective dose now causes emesis or at a given concentration the percentage of dogs showing emesis is increased.

Table 6. Effects of theophylline on emesis threshold.
(Studied in 4 dogs, each before and after theophylline.)

		Controls		Pretreat with Theophylline (I.P.) (25 mg/kg)	
Substance	Concentration	no. trials	% emesis	no. trials	% emesis
Insulin (i.v.)	5 iu/kg	12	0	12	42
	10 iu/kg	24	46	12	66
	15 iu/kg	12	67	12	83
Angiotensin II (i.v.)	0.10 mg/kg	8	0	8	50
	0.15 mg/kg	8	0	8	75
	0.20 mg/kg	8	100	8	100
Apomorphine	.0025	4	0	4	0
	.0050	4	25	4	50
	.0075	4	50	4	75
	.0100	4	100	4	100

Table 7. Effect of IBMX on emesis threshold.

(Each dose tested in each of three dogs before and after IBMX.)

Substance	Concentration	Controls % emesis	Pretreat IBMX (1 mg/kg i.v.) % emesis
Apomorphine	.0010	0	0
	.0015	0	33
	.0025	0	33
	.0050	33	100
	.0100	100	100
Leucine enkephalin	.005	0	0
	.010	0	0
	.015	0	33
	.020	0	33
	.025	33	67
	.050	33	67
	.075	67	100
	.100	100	100

Table 8. Effect of RO-1724 on emetic threshold.

(Each dose tested in each of four dogs before and after RO-1724.)

Substance	Concentration	Controls % Emesis	Pretreat RO-1724 (0.25 mg/kg i.v.) % Emesis
Apomorphine	.0010	0	0
	.0015	0	50
	.0025	0	75
	.0050	75	100
	.0075	100	100
Leucine enkephalin	.005	0	0
	.015	0	25
	.025	25	50
	.050	25	50
	.075	75	50
	.100	100	100

SECTION 4

DISCUSSION

The results of this study provide strong support for the view that at least in the dog the area postrema plays a central role in radiation-induced emesis. Using a dose and protocol found by others to elicit emesis in 100% of animals (10,19) we have confirmed the loss of the emetic response if the area postrema is ablated. It is almost impossible to ablate all of the area postrema without damaging deeper structures, particularly the nucleus tractus solitarius (NTS) which is probably the critical site of projection of area postrema neurons (6). Nonetheless our lesions were essentially complete and caused only limited damage to deeper structures. Since the animals experienced no obvious defects in respiration, circulatory function, chewing or swallowing, all controlled by other neurons in the NTS, we assume that the projection areas of the area postrema were not destroyed.

The demonstration that neurons within the area postrema become electrically active post-irradiation is very strong support for a direct and central role in the mediation of radiation-induced emesis. The neuron illustrated in Figure 2 was fortuitously stable for the full duration of the period where emesis would occur in an unanesthetized animal, and its pattern of activity is exactly as would be expected for the critical central receiving area. At one hour post-irradiation it had a very slow frequency of discharge which accelerated and decelerated to stop after over 4 hours, exactly mirroring the probability of emesis in our unanesthetized animals. Moreover, four other spontaneous units were found in the two dogs studied after irradiation. We had never seen spontaneous units previously, except for those which became spontaneous as a result of our application of excitatory substances.

Given that our and others evidence indicates the necessity of the area postrema in radiation-induced emesis, there are two possible mechanisms of excitation of neurons there. The area postrema receives afferents from numerous sources including fibers

ascending from the spinal cord (36) and a major afferent input from the vagus nerve (23,37). A number of these vagal fibers arise from the stomach wall (38,39), the stomach being in the region of the body most likely to produce emesis when irradiated (3). Thus it is possible that irradiation results in a local irritation or release from some substance which stimulates afferent nerve fiber activity ascending in the vagus and exciting neurons in the area postrema. Alternatively, radiation could cause release of some substance into the circulation which would act directly to excite neurons of the area postrema, since this structure lies outside of the blood-brain-barrier.

The pattern of radiation-induced emesis after bilateral subdiaphragmatic vagotomy is strong support for the humoral mediation of radiation-induced emesis. While our lesions would not have interfered with activity of afferents carried in the sympathetic chain through the spinal cord, the vagus is a much more likely source of significant afferent activity, and is known to carry those afferents responsible for emesis due to local gastric irritation (34). While the deafferented animals had a somewhat longer latency and fewer episodes than the controls, it is not clear whether these differences are significant in so few animals. Nonetheless, it is clear that vagotomized animals exhibited radiation-induced emesis. The spontaneous activity of the area postrema neurons also is most consistent with a direct chemical excitation. Anesthesia, which depresses the behavioral response, acts much more at presynaptic levels than at postsynaptic chemosensitivity sites.

Since these observations strongly suggest that radiation-induced emesis is humorally mediated the question is raised as to what substances could be the mediators. There is no lack of candidates, since neurons in area postrema are excited by such a wide variety of substances. We have pursued two possibilities in these studies, and surprisingly have some support for both. Prostaglandins have a multitude of functions but are known to be synthesized in response to cellular injury in a variety of systems. Prostaglandin levels rise in tissue after irradiation, (25,28) and the

time course of increase is very similar to that of radiation-induced emesis in that there is an initial peak at 1-4 hours, often followed by a later subsequent rise (28). Furthermore, we have shown that several prostaglandins are excitatory on area postrema neurons. Thus the neurons of the area postrema could be excited by circulating prostaglandins, evoking emesis.

Our experiments with dogs pretreated with indomethacin are consistent with this possibility, in that two of seven dogs pretreated did not vomit after irradiation, while all control animals did; the average number of emetic episodes was reduced by about half in the remaining animals pretreated with indomethacin. It is not at all certain that our protocol (indomethacin at 10 mg/kg given 30 min prior to irradiation) would totally block prostaglandin synthesis, and thus this result must be taken as a preliminary result and stimulus for further investigation.

Our results with domperidone confirm and extend the observations of DuBois et al. (30) who first showed that this substance totally blocked radiation-induced emesis in the dog. We found that domperidone not only blocked emesis but also prevented excitation of neurons in the area postrema after irradiation. Since domperidone does not cross the blood brain barrier, one would expect that its action would be either peripheral or at the level of the area postrema. The blockade of area postrema excitation is consistent with this expectation.

Domperidone is a D-2 dopamine receptor antagonist (40) and some have suggested that it also has actions at a previously undescribed dopamine receptor called D-4 (41). The simpliest explanation of its block of radiation-induced emesis would be that the humoral agent is dopamine. This seems unlikely for several reasons. It is not at all clear where the dopamine would come from, since there are few obvious peripheral dopamine stores. One possibility would be mast cells, which are degranulated by ionizing radiation (42) and which while containing principally histamine may also in some species and at some sites contain dopamine and serotonin (43). However if mast

cells were the source, one might expect histamine to be a more potent mediator, since it also excites area postrema neurons (20). The area postrema in a number of species does contain an unusual number of mast cells, although it is not known whether these contain dopamine. However if the efficacy of domperidone were at the level of the D-2 (apomorphine) receptor on area postrema neurons the other D-2 receptor antagonists should be equally effective in preventing radiation-induced emesis. This does not, however, appear to be the case either clinically or in dog studies.

It is possible that the action of domperidone is peripheral, not at the level of the area postrema and that in some fashion it prevents the release of another humoral agent(s). Domperidone is not very effective in preventing radiation-induced emesis in the monkey (44). Its dramatic action in the dog is nevertheless a lead with which to pursue mechanisms.

Prostaglandins have been shown to cause emesis in both humans and animals (45-48). They are released from tissue following exposure to radiation (49). Our results suggest the possibility that prostaglandins released into the blood stream may induce emesis by directly exciting neurons of the area postrema in a manner similar to that of other neurotransmitters.

The possibility that prostaglandins may function as neurotransmitters or modulators has received little general attention in spite of numerous reports documenting effects in a variety of systems. For example, Alanzino et al., (50), reported that prostaglandin E_1, E_2 and $F_2\alpha$ had both excitatory and inhibitory responses on cat brain stem neurons. Similar results were obtained by Siggins et al., (51), in recordings from cerebellum. Prostaglandins are released from brain tissue on stimulation (52). Stomach muscle contracts to prostaglandins, and prostaglandin E_1 is released when nerves to the stomach are electrically stimulated, (53), and this release can also be stimulated by serotonin (54).

Prostaglandins have been proposed as mediators of the peripheral somatostatin-induced inhibitor of gastric secretion, and E_2 and $F_2\alpha$ have also been proposed to have central actions in the control of gastric secretion (55,56). Presynaptic prostaglandin receptors have been proposed to explain the effects these substances have on transmitter release from the autonomic nervous system (57) and on smooth muscle innervation (58,59). It has been proposed that the principal mechanism of action of prostaglandins causing direct excitation or alteration of transmitter release is through regulating calcium currents across the membrane (60,61). Prostaglandins may mediate the rebound contraction triggered in smooth muscle of the gut to activation of the non-adrenergic, non-cholinergic nerves (62). A variety of behavioral effects of prostaglandins have also been described (63).

Among the questions remaining unanswered by our study are whether there are distinct receptors for each of the different active prostaglandins or whether all are acting at a common receptor. Coleman et al. (64) have proposed that five different prostaglandin receptors exist, one for each of the natural prostaglandins, D_2, E_2, $F_{2\alpha}$, I_2 and thromboxan A_2, but suggest that there may be extensive cross-reactivity, with lower potency. Some specificity must exist since we found no responses to prostaglandin A_2, D_1, and D_2. The mechanism whereby the prostaglandins mediate their excitatory actions is also unknown, and is of particular interest since their responses are similar to those of apomorphine, biogenic amines and several neuropeptides (20). Further study of the actions of prostaglandins on neurons will be of interest, since our results corroborate the fact that various prostaglandins have direct actions on neurons, and also suggest that they may mediate a complex behavioral response such as emesis.

Our results constitute one of the first demonstrations of direct excitation of neurons by insulin, thus supporting the suggestion that insulin may serve a transmitter function in the mammalian brain. Similar results have been reported in abstract by Waldbillig (65) from studies of rat area postrema. The rat preparation

has some different properties, especially in that the neurons are spontaneously active, and the rats do not exhibit emesis. Nevertheless our results are in good agreement with regard to the action of insulin. The only other report of insulin-mediated excitation that we are aware of is the report by Oomura and Kita (66) that neurons in the lateral hypothalamus which are inhibited by glucose are excited by insulin. Although their records of insulin excitation are very clear it is not certain whether this action is a direct action of insulin or is mediated by removal of glucose inhibition.

Nausea and vomiting are side effects of the hypoglycemia which accompanies excessive insulin administration in humans, but the action has been presumed to be indirect through the autonomic nervous system (67). Our results provide evidence that insulin may induce emesis by direct excitation of area postrema neurons. The observation that insulin can excite central mammalian neurons, when considered in the context of its presence and distribution in brain and the reports of inhibitory actions on some neurons (68) is consistent with the possibility that insulin may have a transmitter function quite independent of other hormonal roles.

The remarkable feature of area postrema neurons is that while small in size these cells have receptors for a great number of substances. Secondly, the responses to most of them (all of the excitatory substances reported here except glutamate) have the same characteristics of being relatively long in latency, slow in frequency of discharge and long in duration. There are several possible explanations for these findings.

It is possible that the excitatory responses we have recorded are not direct, but rather are synaptically mediated by small neurons from which we cannot record because of their size. It is known that there are many neurons in the area postrema, especially in the outer layers, while those from which we have primarily recorded were usually located 500-700 μM from the surface (69,70). If the primary receptors

were on smaller neurons, where specific receptors were located for the variety of excitatory substances, the excitation of the neurons we have studied could be due to a single transmitter release on the larger neurons from a variety of smaller cells. This possibility cannot be excluded but seems very unlikely since the ionophoresis is localized and it seems unlikely that excitation of a few small neurons in the close region of the cell under study would be sufficient to drive it.

It is conceivable that these neurons have some sort of primitive receptor which is not very selective and responds to a great variety of substances. This, however, also seems unlikely. The neurons do not respond to everything, as indicated by the lack of response to nicotine and somatostatin. Furthermore, in almost every neuron studied we could get responses to some of the substances in the ionophoretic pipette but not to others. In addition, our previous behavioral studies (22) have shown that for at least three of the receptors which mediate emesis (and we believe that those receptors are the ones reported here in the area postrema, since emesis from these substances was abolished by area postrema ablation) it was possible to pharmacologically block the response selectively. Thus emesis due to apomorphine was blocked by domperidone without effect on emesis due to angiotensin II and leucine enkephalin, while naloxone blocked the leucine enkephalin emesis without altering that to the other two substances. These observations are compatible with the idea that the receptors for at least these three substances, and by extrapolation, those to the others as well, are distinct entities and agonist specific, as elsewhere in the nervous system.

It is also possible that the 20 or so receptors which mediate the slow excitatory responses are all specific, but that all of the receptors are coupled to the same type of ionic channel. There are many precedents for this possibility. Especially in invertebrate neurons most of the conventional transmitters may, on different neurons, show responses which elicit either Na^+, Cl^- or K^+ conductance increase responses (71). Often one neuron will have the same ionic response to more than one

transmitter (72,73) but the responses are pharmacologically distinct. In such a situation one can sometimes find pharmacologic agents which act not at the receptor but at the channel, and in this case all of the responses will be blocked (74). This situation cannot be eliminated as the mechanism for area postrema neurons, although it seems to be a somewhat inefficient design when dealing with so many receptors on such small neurons.

A third possibility is that the receptors for all of these substances are distinct, but all activate a common second messenger. This mechanism has been demonstrated in a variety of systems, and at least cyclic AMP, (75) cyclic GMP (76) and Ca++ (77) have been proposed to function in this fashion. Since many of the actions of the cyclic nucleotides are related to activation of specific protein kinases and consequent stimulation of protein phosphorylation, it is possible that a variety of other mechanisms exist which could function as second messengers. One of the first clear demonstrations of cyclic AMP functioning as a second messenger was the study of epinephrine and adenosine receptors on turkey erythrocytes (78). Both substances were found to act by stimulation of an adenylate cyclase. These authors suggested, and subsequent evidence has supported the postulate, that a single cyclase mediated these effects, and that the receptors and the cyclase exist independently in the membrane, with each specific receptor binding to and activating the cyclase with the assistance of a special binding protein after agonist binding to its receptor.

Our evidence supports the possibility that the slow excitatory responses of area postrema neurons are mediated by cyclic AMP synthesis, probably through a common adenylate cyclase similar to that reported above in turkey erthrocytes. The neurons respond to 8-bromo cyclic AMP, a relatively membrane permeable derivative of cyclic AMP. Moreover, the neurons are also excited with a similar pattern of action by forskolin, known to be a direct activator of adenylate cyclase (35). Cyclic GMP did not have similar actions.

The behavioral experiments also are consistent with this explanation. While attempts to achieve total body inhibition of phosphodiesterase may seem somewhat crude, systemic administration of both the methyl xanthine (theophylline and IBMX) and non-methyl xanthine (RO-1724) phosphodiesterase inhibitors clearly shifted the threshold sensitivity of all of the substances tested. These studies, by themselves, do not prove the hypothesis, but when coupled to the ionophoretic investigations are strong support for the possibility that phosphodiesterase inhibitors shift the threshold agonist dose required to trigger emesis by slowing the rate of breakdown of cyclic AMP which is induced by the agonists.

There are some problems with the proposal that this is a general mechanism for all of these substances, since some of the active substances have not previously been found to activate adenylate cyclase. This is particularly true for apomorphine and dopamine, in that while there is a dopamine D1 receptor which is known to be coupled to adenylate cyclase (79), the receptor in the area postrema is pharmacologically D2 (80), and this type is not supposed to be cyclic AMP dependent. Insulin is also somewhat of a problem, since insulin in most other systems is an inhibitor of adenylate cyclase (81) and an activator of phosphodiesterase (82). Cyclic AMP activation has been demonstrated at least by norepinephrine, dopamine, serotonin, histamine, glutamate, prostaglandin E, (83) muscarinic acetylcholine (84) epinephrine (85) vasoactive intestinal polypeptide (86) and glucogon (87) receptors in other systems, however. It seems likely that cyclic AMP mediates all of the slow agonist actions we have observed, but biochemical confirmation would be nice although difficult to obtain in such a small brain area of the dog. The possible association of so many different specific transmitters with the same postsynaptic mechanism is consistent with the hypothesis put forward by Swann and Carpenter (72) that transmitter receptors and ionophores are independent elements in neuronal membranes, and can be arranged in any combination.

The electrophysiological correlates of transmitter activation of cyclic AMP synthesis have in some systems been reported to be a slow depolarization, associated with a decrease in membrane conductance to K+ (88). This would be a reasonable ionic mechanism to expect to apply here, and is a testable hypothesis.

Another remarkable characteristic of these responses is that the percentage of responsive cells is so great. On the average, about 50% of the neurons studied responded to the excitatory substances (with the obvious exception of glutamate, which was used to find the cells and therefore may have induced a selection of its own). Given the limitations of the technique of extracellular recording and ionophoresis through a multiple barrel electrode, this rate of responsiveness is so high as to suggest that all of the cells have all of the receptors. Certainly the effect of ionophoresis must have been subthreshold on occasion, particularly since some cells must have been oriented with regard to the electrode such that substances from some barrels were less directly applied that was the case for others.

These considerations suggest that on the basis of the transmitter receptors we have recorded from a single population of neurons. This also is very surprising, since there are not only a variety of cells types in the area postrema (69,70) but it is very clear that on the basis of the transmitters which the neurons in the area postrema contain (89) there are a variety of cell types. While it is clear that we have not recorded from all of the neurons in the area postrema, those we have studied are probably the large neurons in the deeper layer (69) which constitute the principal output pathway (70). Possibly these cells are homogeneous in terms of receptors, since they presumably are all responding to the same emetic stimuli, but use different transmitters to transmit different information to higher nervous system levels.

There is considerable controversy over the roles of the area postrema. Its role in emesis is best established, and Borison (90) believes that that is its only function. However, a variety of other studies have led to the conclusion that the area postrema also is involved in water regulation through angiotensin II receptors (91), food intake through cholecystokinin receptors (92,93), and EEG synchronization through serotonin receptors (94). Our results do not speak to this issue directly, except in that most of the substances for which we find excitatory receptors in the area postrema are also emetic.

As summarized in Table 2, those substances which are excitatory on area postrema neurons have with few exceptions been shown to be emetic. Certainly it requires a relatively high systemic concentration of peptides and hormones to trigger emesis, but then it is likely that the purpose of the receptors is to respond to unphysiologic circulating levels of active substances. The correlation of substance-induced emesis with the presence of excitatory receptors in the area postrema is support for the view that these receptors are those which mediate emesis. Of course the output neurons of the area postrema may project to other places than the motor emetic center, and elicit other kinds of behavior than emesis. However it is hard to see how activity of the neurons we have recorded from can trigger actions specific to any one particular transmitter, peptide or hormone, since all of the neurons have receptors for so many substances. Since no other behavioral response is so general to this group of excitatory substances our results are in general agreement with Borison's idea that emesis is the primary function of this structure. It may be that the area postrema, like the other circumventricular organs, allows some substances such as angiotensin II and cholecystokinin, to pass through its vasculature (95,96) and to act at sites such as the nucleus tractus solitarious to mediate other specific actions. It is also possible that the small neurons in the more superficial zones of the area postrema might subserve other functions, but we have not been able to record from these neurons.

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SPONSORING ORGANIZATION: _____

CONTRACTING OFFICER OR REPRESENTATIVE: _____

SIGNATURE: _____